DIGESTIVE ENZYMES OF THE ECHIUROID, OCHETOSTOMA ERYTHROGRAMMON

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Apart from the observation of Gislén (1940) that the brown juice from the midgut of *Echiurus echiurus* contained a proteolytic enzyme, an amylase and an esterase, work on the enzymes of echiuroids is lacking.

In the present study observations were made on the digestive enzymes and their distribution along the gut of *Ochetostoma erythrogrammon*.

Materials and Methods

Specimens of *Ochetostoma erythrogrammon* from the intertidal muddy sand of the west coast of Singapore Island were starved in filtered sea water in the laboratory for at least four weeks, to clear the gut of faecal pellets and to allow the digestive fluid to accumulate.

The pH of the gut fluid was determined by mixing it with an equal quantity of indicator solution and matching the color formed against a series of standards made up of indicator solution and buffers of known pH. The indicators used were brom-thymol blue and thymol blue.

A high incubation temperature increases both the catalytic activity and the rate of inactivation of an enzyme (Dixon and Webb, 1958). Because of the small quantity of gut fluid and enzyme extracts, temperatures of 35° C. and 40° C. were employed to produce sufficient digestion products for titration within three hours. Presumably, these temperatures, being near to the maximum temperature experienced by *Ochetostoma* in nature, did not greatly inactivate the enzymes. At ebb tide on a windless sunny day, the shallow water of tide pools bathing these animals often reached a temperature of 37° C.

In the proteolytic enzyme experiments 0.1 ml. of gut fluid was incubated at 40° C. with a mixture of 1 ml. Clark and Lubs buffer solution and 1 ml. of 6% gelatine. The proteolytic activity was determined by titrating the carboxyl groups released with 0.01 N alcoholic potassium hydroxide solution and using 1% alcoholic thymolphthalein solution as indicator (Tauber, 1950). Control experiments at all pH values were carried out. Tests on egg albumen, casein, fibrin, nylon fibers and spongin fibers were made.

In the amylase experiments 0.15 ml. of gut fluid was incubated at 35° C. with a mixture of 0.5 ml. Sorensen's phosphate buffer and 1 ml. of 2% soluble starch solution. The amylolytic activity was determined by recording the time taken by the enzyme-substrate mixture to become colorless when tested with light-yellow solution of iodine in 2% potassium iodide solution.

For other carbohydrase experiments 0.15 ml. of gut fluid was incubated at 40° C. with 5 ml. of Sorensen's phosphate buffer at pH 7.0 with 20 ml. of 2%

inulin solution or 5% solutions of lactose, maltose or sucrose. The enzymic activity was determined by titration with Barfoed's solution or Benedict's solution. Phenylhydrazine tests were carried out for confirmation. Other tests included cellulose fibers (from filter paper) and glycogen as substrates.

Esterase activity at various pH values was determined by titrating with 0.01 N alcoholic potassium hydroxide digests containing midgut fluid or gut-wall extract and the substrate benzyl n-butyrate (Tauber, 1950). Olive oil and ethyl butyrate were also used as substrates in other esterase tests (Hawk, Oser and Summerson, 1947).

Results

In this study the gut is subdivided anteriorly simply into the muscular pharynx, with radiating muscular strands, and the less muscular oesophagus, which swells up posteriorly into a crop. This is encircled by a loop of blood vessel and is succeeded by the foregut, which has throughout its length a ciliated groove. The midgut which follows has a siphon. The hindgut has a ciliated groove but no siphon and ends in a muscular bulbous cloaca.

Freshly collected specimens had accumulations of light-yellow to purplish fluid at various regions of the midgut. After starvation in filtered sea water for

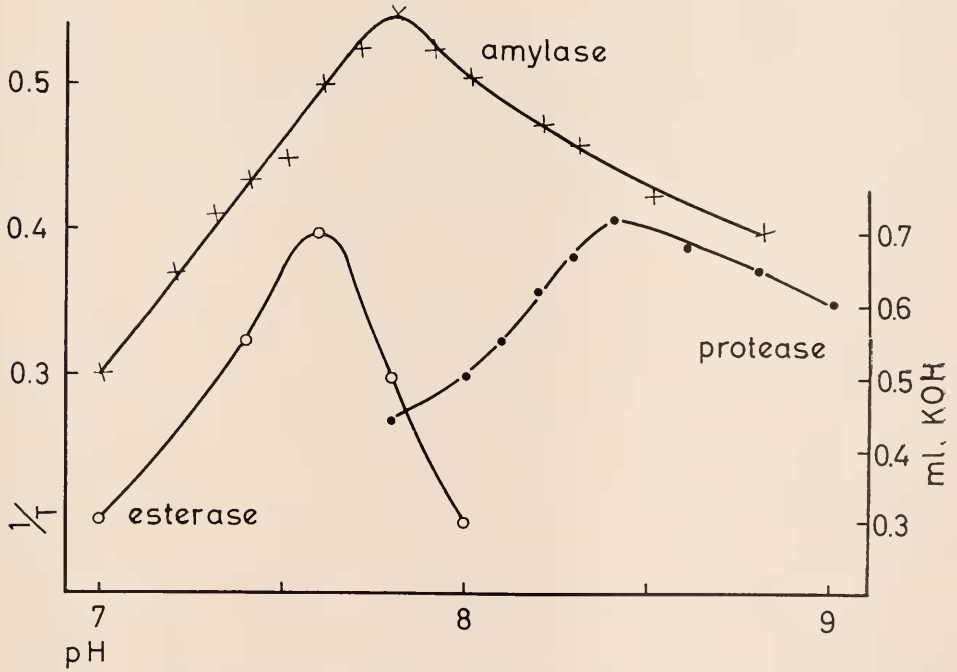

Figure 1. The pH activity curves of amylase, protease and esterase from the midgut fluid of *Ochetostoma erythrogrammon*. 1/T = reciprocal of time taken by the amylase-starch mixture to become colorless with iodine; ml. KOH = titer of 0.01 N KOH per 0.1 ml. digest of protease-gelatine and per ml. digest of esterase-benzyl n-butyrate.

several weeks the midgut fluid seemed to increase. Occasional accumulation of fluid also occurred in the adjoining parts of both the hindgut and the foregut.

By pooling the midgut fluids of 18 small, 16 medium, 10 large and 7 large starved specimens separately, averages of 0.09, 0.14, 0.23 and 0.33 ml. of fluid per worm, respectively, were obtained. The pH of midgut fluid from 10 starved specimens had a range of 7.0–8.2 with an average of 7.5, the lighter-colored fluid tending to be more alkaline.

The midgut fluid, extracts of the hindgut, midgut and foregut showed proteolytic activity, which decreased in that order per unit weight of gut tissue with gelatine as substrate. Extracts of pharynx, oesophagus and crop had no proteolytic activity. The pH activity curve of the proteolytic enzyme in the gut fluid shows a maximum at pH 8.4 (Fig. 1). Other proteins digested include casein, egg albumen and fibrin. Spongin and nylon fibers were not digested.

The midgut fluid showed amylolytic activity which reaches a maximum at pH 7.8 (Fig. 1). The starch solution undergoing this enzymic hydrolysis, when tested at intervals with iodine test solution, showed a change from blue through shades of mauve and pink to colorlessness, indicating a diminution in the size of the starch molecules to colorless achroodextrins and maltose. This was identified as maltosazone with the phenylhydrazine test. Glycogen was also digested. The amylolytic activity of 5% extracts of the midgut, foregut, crop and hindgut decreased in that order at pH 7.8. The extracts of the pharynx and oesophagus showed no amylolytic activity.

The midgut fluid, extract of midgut and extract of hindgut contained an esterase which hydrolyzes olive oil, benzyl n-butyrate and butyl acetate. In an individual specimen the esterase activity decreased in the following order: entire midgut fluid, extract of entire hindgut, extract of entire midgut. Extracts of the pharynx, oesophagus, crop and foregut showed no esterase activity (Table I). With benzyl n-butyrate as substrate the esterase activity reached a maximum at about pH 7.6 (Fig. 1).

TABLE I

Wet weight, and relative strength of digestive enzymes, of the various gut regions of Ochetostoma erythrogrammon

	Pharynx	Oesophagus and crop	Foregut	Midgut	Hindgut	Midgut fluid
Average wet weight of gut (minus gut contents) of 5 large specimens in mg.	0.7	1.3	4.8	41	53	
Protease	—*	—	+*	++	++	+++
Esterase	—	—	—	+	++	+++
Amylase	—	++	+++	++++	+	+++++
Maltase	—	—	—	—	+	+ or −

* — indicates absence; +, presence, with more crosses for greater strength.

Hindgut extract and some samples of midgut fluid contained a weak maltase. The midgut fluid had no inulase, cellulase, sucrase or lactase.

Discussion

Ochetostoma erythrogrammon is a detritus-feeder (Chuang, 1962). Its midgut fluid contains a protease, esterase and amylase as in *Echiurus echiurus* (Gislén, 1940). Brasil (1904), Nicol (1931) and Dales (1955) also found these in extracts of the gut wall of the polychaetes *Pectinaria koreni, Sabella pavonina* and *Amphitrite johnstoni,* respectively. The protease and amylase are strong in *O. erythrogrammon* and the three polychaetes mentioned above, while in *E. echiurus* both amylase and esterase are weak (Gislén, 1940).

The distribution and relative strength of the important enzymes along the alimentary canal of *O. erythrogrammon* are summarized in Table I. Protease is more extensively distributed than esterase. The most widespread enzyme is the amylase, which is absent only in the short length of the gut anterior to the foregut; its concentration is least in the hindgut, where maltase occurs. There is thus a localization of enzymes splitting higher and smaller products of starch in this animal as in vertebrates. In *Amphitrite johnstoni* the protease, amylase and esterase are confined to the fore-stomach and fore-intestine (Dales, 1955), while in *Pectinaria koreni* amylase occurs only in the most anterior part of the midgut, and protease, in the second part of the midgut (Brasil, 1904). Digestive enzymes are therefore more widely distributed along the alimentary canal of *O. erythrogrammon* than in the polychaetes.

Amylase and maltase mostly occur together in invertebrates (Vonk, 1937). This is so for the hindgut of *O. erythrogrammon* but not for the oesophagus, crop, foregut and midgut (Table I). Some samples of midgut fluid contain maltase, others not, indicating either the secretion of maltase in the midgut of some specimens or the escape of hindgut maltase into the midgut lumen, which continues uninterruptedly into the hindgut lumen without an intervening sphincter. The amylase in both *O. erythrogrammon* and the polychaete *S. pavonina* hydrolyzes both starch and glycogen as amylases from other sources do (Tauber, 1950).

The pH of the gut contents varies from one end of the alimentary canal to another. In *O. erythrogrammon* it rises from 7.6 in the crop to a maximum of about 8.2 in the midgut and then falls to 8.0 in the hindgut. In *S. pavonina* it rises from 6.8 in the first tenth to thirtieth segment to a maximum of between 8.0 and 8.4 at about the one hundredth segment, falls suddenly to 7.0 and then more slowly to 6.0 (Nicol, 1931). In *Amphitrite johnstoni,* however, it dropped from 7.0 in the oesophagus to about 6.0 in the fore-stomach, this minimum being maintained throughout the following region until the hind-intestine, from where it rises from 6.2 to a maximum of 7.2 at the rectal end (Dales, 1955). Dales also found that starved worms had a higher pH throughout the length of the gut. The higher values of the gut contents in *O. erythrogrammon* are associated with higher pH optima for the chief digestive enzymes. The pH optima of protease, amylase and esterase in *O. erythrogrammon* lie at 8.4, 7.8 and 7.6, respectively, against 8.0, 6.8 and 7.4 for *S. pavonina* (Nicol, 1931). The activity of the protease on gelatine in *O. erythrogrammon* decreases more slowly on the alkaline

side of the pH range than on the acid side as in *S. pavonina* (Nicol, 1931). The esterase in both is active over a narrow pH range.

The specificity of the enzymes of *O. erythrogrammon* is similar to that of the polychaete *S. pavonina.* In both the amylase digests both starch and glycogen, the protease hydrolyzing gelatine, casein, fibrin and egg albumen, the esterase splitting neutral fat, such as olive oil, and esters, such as ethyl butyrate (Nicol, 1931).

The midgut and the hindgut in *O. erythrogrammon* constitute the greater part of the alimentary canal (Table I), as in *Echiurus echiurus* (Spengel, 1880), *Urechis chilensis* (Seitz, 1907) and *Urechis caupo* (Fisher and MacGinitie, 1928). They also contain many digestive enzymes. Fluid accumulates in the midgut as in *E. echiurus* (Greef, 1879), and occasionally also in the adjoining parts of the foregut and hindgut. The long coiled midgut, with its wide lumen often distended with a large amount of sand and fluid, is presumably the most important site of digestion in *O. erythrogrammon,* as Greef (1879) suggested for *E. echiurus.* The hindgut of *Ochetostoma,* with its multitude of enzymes, may also play an important role in digestion. The foregut may be less important and the more anterior regions of the gut presumably do not contribute much to actual digestion of food because of their short length and poor enzyme content.

The midgut fluid of *O. erythrogrammon* has a high concentration of digestive enzymes. For instance, 0.05 cc. of midgut fluid has an amylolytic activity of 30 mg. of midgut wall (wet weight) and gelatine-hydrolyzing power of 200 mg. of midgut wall. Similarly, the midgut fluid is richer in esterase than the midgut or hindgut wall. Seitz (1907) found secretory granules in the epithelial cells of the midgut in *U. chilensis.* Presumably, the midgut fluid of *Ochetostoma* represents an accumulation of continuous enzyme secretion over the long period of starvation, and is therefore more potent than extracts of the gut wall, which contain only what was in the cells at the time of extraction. Presumably, *O. erythrogrammon* digests its food mainly extracellularly in the midgut lumen. Largely on histological grounds, Dales (1955) also presumed extracellular digestion in *Amphitrite johnstoni.*

From the digestive enzymes of the midgut fluid, *O. erythrogrammon* appears able to digest food of both animal and plant origin from the detritus collecting on the wall of the burrow and on the surface mud outside (Chuang, 1962). However, the lack of such carbohydrases as cellulase, lactase, sucrase and inulase must considerably reduce its ability to utilize detritus of plant origin. Nicol (1931), finding no lactase, maltase, cellulase or sucrase in *S. pavonina,* also concluded that plant detritus was probably not utilized by *Sabella.*

Summary

1. The digestive enzymes of *Ochetostoma erythrogrammon* were investigated. They include a strong amylase, a strong protease, a moderately strong esterase and a weak maltase. No cellulase, sucrase, lactase or inulase were found.

2. The midgut fluid accumulated during starvation in the lumen of the entire midgut. It proved a richer source of digestive enzymes than extracts of the various regions of the gut. Amylase occurred along the entire gut wall except

the pharynx. Protease occurred in the foregut, midgut and hindgut, esterase in the midgut and hindgut, and maltase only in the hindgut.

3. Both amylase and protease are active over a wide range of pH values, but the esterase has a more restricted range. The pH optima of these lie on the alkaline side of neutrality, with the protease having the highest pH optimum of 8.4 for the substrate gelatine.

4. The gut contents had an alkaline reaction, the pH rising from the crop to a maximum in the midgut and then dropping slightly in the hindgut. The pH of the midgut fluid in starved specimens varied between 7.0 and 8.2.

5. Digestion in *O. erythrogrammon* is presumably extracellular, occurring chiefly in the midgut. The ability of *Ochetostoma* to utilize plant detritus must be considerably reduced by its lack of carbohydrases other than amylase and a weak maltase.

6. The enzymes of *O. erythrogrammon* are discussed and compared with those of polychaetes.

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